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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/607,903	06/27/2003	Gjalt W. Huisman	MBX 025 DIV CON	7536

23579 7590 01/24/2008
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EXAMINER

HUTSON, RICHARD G

ART UNIT	PAPER NUMBER
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1652

MAIL DATE	DELIVERY MODE
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01/24/2008

PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 10/607,903	Applicant(s) HUISMAN ET AL.	
	Examiner Richard G. Hutson	Art Unit 1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 24 October 2007.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-8, 11-19 and 21 is/are pending in the application.
- 4a) Of the above claim(s) 11, 12 and 14-17 is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-8, 13 and 18 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- * See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 10/24/2007 has been entered.

Applicant's cancellation of claims 9, 10, 20, 22 and 23 and amendment of claims 1, 2, 7, 11, 12, 14-17, 19 and 21, in the paper of 10/24/2007, is acknowledged. Claims 1-8, 11-19 and 21 are still at issue and are present for examination.

Applicants' arguments filed on 10/24/2007, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claims 11, 12, 14-17, 19 and 21 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

Claim Objections

Claims 1 and 7 are objected to because of the following informalities:

Claims 1 and 7 each recite "when the bacteria is lyzed by osmotic shock". This should be "when the bacteria is lysed by osmotic shock.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-8, 13, 18 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

This rejection was stated in the previous office action as it applied to previous claims 1-10, in response to this rejection applicants cancelled claims 9, 10, and amended claims 1, 2 and 7 and traverse the rejection as it applies to the newly amended claims.

Applicants traverse this rejection on the basis that applicants claims define a bacterial strain for production of a fermentation product selected from the group consisting of antibiotics, organic acids, amino acids, proteins, vitamins, polyhydroxyalkanoates and polysaccharides, wherein the bacterial strain is genetically modified to express a heterologous nuclease gene, wherein the nuclease gene product

is secreted into the periplasmic space and released when the bacteria is lysed by osmotic shock. Applicants submit that using bacterial strains, nucleases and genetic engineering techniques which are known in the art the present Application describes how to produce novel organisms that secrete nuclease into the periplasmic space as claimed.

Applicants submit that bacterial strains, such as *Ralstonia*, *Aeromonas*, *Azotobacter*, *Burkholderia*, *Comamonas*, *Methylobacterium*, *Paracoccus*, *Pseudomonas*, *Rhizobium*, and *Zoogloea*, have been sold by the American Type Culture Collection in Rockville, MD and used in school laboratories and commercial fermentation facilities for many years and all are well known to be amenable to typical manipulations of bacterial genetics, allowing the use of broad host range cloning vectors as transforming vehicles for a nuclease gene of interest .

Applicant's amendment and complete argument is acknowledged and has been carefully considered, however, is found nonpersuasive for the reasons previously made of record and repeated herein.

While knowledge of one skilled in the art is relevant to meeting the written description requirement, applicant is reminded that the instant rejection is based on a lack of written description, not a lack of enablement. Regardless, applicants argument is not persuasive because while bacterial strains and nuclease genes for use in the making of the claimed mutants, as well as methods to produce variants of a known sequence such as site-specific mutagenesis, random mutagenesis, etc., are well known to the skilled artisan, producing variants as claimed by Applicants (i.e., a bacterial strain

comprising any heterologous nuclease gene such that expression or modification is an amount effective to degrade nucleic acid) requires that one of ordinary skill in the art know or be provided with guidance for the selection of which of the infinite number of variants have the claimed property. Without such guidance one of ordinary skill would be reduced to the necessity of producing and testing all of the virtually infinite possibilities. This would clearly constitute undue experimentation. While the statute is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Such guidance has not been provided in the instant specification as the specification discloses only the species of the claimed genus encompassed by *P. putida*, *R. eutropha* and *E. coli*, expressing the heterologous *Staphylococcus aureus* nuclease gene, *nuc*, which is insufficient to put one of skill in the art in possession of the attributes and features of all species within the claimed genus.

The genus of bacterial strains that are claimed is a large variable genus comprising any bacterial strain comprising any heterologous nuclease gene. While one of skill in the art may have used routine experimentation to laboriously arrive at those strains encompassed by the instant claims which comprise a genetic modification of a heterologous nuclease gene, applicants were clearly not in possession of such strains, as applicants do not describe even a small portion of the encompassed bacterial strains. Therefore, one skilled in the art cannot reasonably conclude that the Applicant had possession of the claimed invention at the time the instant application was filed.

Applicants submit that the application contains examples demonstrating that one can genetically engineer known strains of bacteria with known nuclease genes to make a product as claimed, however as discussed above this rejection is not based on a lack of enablement, but rather a lack of written description, and as discussed above, applicant is reminded that applicant examples merely teach the isolation of the *Staphylococcus aureus* nuclease gene *nuc* and the integration of this isolated heterologous nuclease gene into three different bacterial strains, *P. putida*, *R. eutropha* and *E. coli*.

As stated previously and above, the specification fails to describe representative species of these bacterial strains by any identifying structural characteristics or properties other than the functional characteristics recited in the claims, for which no predictability of structure is apparent. There is no disclosure of any particular structure to function/activity relationship in the disclosed species with respect to those heterologous nuclease genes or those genetic modifications of homologous nuclease genes such that expression or modification is in an amount effective to degrade nucleic acid so that recovery of a product is enhanced.

The genus of bacterial strains that are claimed is a large variable genus comprising any bacterial strain comprising any heterologous nuclease gene, wherein said nuclease gene product is secreted into the periplasmic space and released when the bacteria is lyzed by osmotic shock. The specification discloses only the species of the claimed genus encompassed by *P. putida*, *R. eutropha* and *E. coli*, expressing the heterologous *Staphylococcus aureus* nuclease gene, *nuc*, which is insufficient to put

one of skill in the art in possession of the attributes and features of all species within the claimed genus. Therefore, one skilled in the art cannot reasonably conclude that the Applicant had possession of the claimed invention at the time the instant application was filed.

Applicants comments with respect to the rationale for the inclusion of claims 2-5 and 7, drawn to those bacterial strains capable of growth to cell densities of at least 50g/l (claim 2), those bacterial strains which produce a polyhydroxyalkanoate to a level of at least 40% of its dry weight (claim 3), those bacterial strains for use in an aqueous process to manufacture poly(3-hydroxyalkanoate) which is essentially free of nucleic acids (claim 4), those bacterial strains for use in a process for making any of a number of different polysaccharides (claim 5), or those bacterial strains selected from the group consisting of *Ralstonia eutropha*, *Methylobacterium organophilum*, *Methylobacterium extorquens*, *Aeromonas caviae*, *Azotobacter vinelandii*, *Alcaligenes latus*, *Pseudomonas oleovorans*, *Pseudomonas fluorescens*, *Pseudomonas putidas*, *Pseudomonas aeruginosa*, *Pseudomonas acidophila*, *Pseudomonas resinovorans*, *Escherchia coli*, and *Klebsiella* (claim 7) are acknowledged. Claims 1-5 and 7 as well as the additional dependent claim 8 are included in this rejection for the same rationale that independent claim 1 has been rejected above, as none of these dependent claims sufficiently limit independent claim 1 such that the claimed genus was considered to be adequately described. Each of the dependent claims are drawn to those bacterial strains comprising any heterologous nuclease gene as well as any genetic modification of any such that expression or modification is an amount effective to degrade nucleic

acid so that recovery of a product is enhanced. Therefore, one skilled in the art cannot reasonably conclude that the Applicant had possession of the claimed invention at the time the instant application was filed.

Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

Claims 1-8, 13, 18 are further rejected under this statute on the basis that the newly added recitation of "the nuclease gene product is secreted into the periplasmic space and released when the bacteria is lysed by osmotic shock" and those methods drawn to this subgenus of methods are not supported by applicants specification at the time of filing and is thus considered new matter.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 2, 4, 5, 6 and 8 are rejected under 35 U.S.C. 102(b) as being anticipated by Liebl et al. (J. Bacteriology 174(6): 1854-1861 (1992)).

This rejection was stated in the previous office action as it applied to previous claims 1, 2, 4, 5, 6 and 8. In response to this rejection applicants amended claims 1 and 2 and traverse the rejection as it applies to the newly amended claims.

Applicants traverse this rejection on the basis that Liebl teaches *Staphylococcal* nuclease (SNase) expression by various *C. glutamicum* strains, wherein the *C. glutamicum* transgenic strain is to be used for investigating protein export and processing. Applicants submit that Liebl et al. is concerned with investigating protein secretion in *C. glutamicum* and that Liebl et al. does not disclose the claimed bacterial strain which is genetically modified to express a heterologous nuclease gene, wherein the nuclease gene product is secreted into the periplasmic space. Applicants submit that *C. glutamicum* is a gram positive bacterium, which does not have a periplasmic space (see for example, Sakamoto, et al., Microbiology, 147:2865-2871 (2001), at page 2865, right column). Thus, Liebl cannot anticipate the claimed bacterial strain. Therefore, applicants submit that claims 1-8 are novel over Liebl.

Applicant's amendment and complete traversal is acknowledged and has been carefully considered, however, is not found persuasive for the reasons previously stated and repeated herein. First, while it is acknowledged that *C. glutamicum* is a gram positive bacterium, and traditionally a periplasmic space has not been identified as being associated with gram positive bacteria, this is not now held to be true, as a number of scientists have identified a periplasmic space in a number of Gram positive bacteria (Zuber et al. J. Bacteriology, Vol. 188, No. 18, pp 6652-6660, 2006). Thus

applicant's argument that Gram positive bacteria have no periplasmic space is not found persuasive.

Second, in addition to using *C. glutamicum*, as described previously and above, Liebl et al. used the gram-negative bacterium, *E. coli*, for much of the previously described work. Thus as the gram negative bacterium, *E. coli*, clearly has a periplasmic space, those bacterial strains taught by Liebl et al. anticipate applicants claimed strains for these and the previously stated reasons.

It is noted that each of the bacterial strains produced by Liebl et al. would secrete the nuclease gene product into the periplasmic space and the product would be released upon lysis by osmotic shock.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claim 1-8, 13, 18, are rejected under 35 U.S.C. 103(a) as being unpatentable over Greer et al. (WO 94/10289 (1994)), Atkinson et al. (Biochemical Engineering and Biotechnology Handbook 2nd edition, Stockton Press: New York, 1991) and Lee et al. (Production of poly(hydroxyalkanoic Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).acid, Adv. Biochem. Eng. Biotechnol.

52:27-58, 1995), in view of Liebl et al. (J. Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).

This rejection was stated in the previous office action as it applied to previous claims 1-10, in response to this rejection applicants cancelled claims 9, 10, and amended claims 1, 2 and 7 and traverse the rejection as it applies to the newly amended claims.

It is noted that each of the bacterial strains made obvious, would secrete the nuclease gene product into the periplasmic space and the product would be released upon lysis by osmotic shock.

Applicants traverse this rejection by first stating applicant's interpretation of the legal standard, followed by applicant's interpretation of what each of the cited references teaches.

Applicants traverse this rejection on a number of different basis. In response to applicant's arguments, many of which are made against the references individually, Applicant is reminded that one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Applicants traverse this rejection as the above rejection on the basis that applicants submit that Liebl does not disclose a bacterial strain genetically modified to express a nuclease gene and secrete the gene product into the periplasmic space on the basis that Liebl discloses expression in a Gram positive bacteria, which does not

have a periplasmic space. Applicant's initial submission is not found persuasive for the same reasons stated above under the rejection based upon anticipation. While it is acknowledged that *C. glutamicum* is a gram positive bacterium, and traditionally a periplasmic space has not been identified as being associated with gram positive bacteria, this is not now held to be true, as a number of scientists have identified a periplasmic space in a number of Gram positive bacteria (Zuber et al. J. Bacteriology, Vol. 188, No. 18, pp 6652-6660, 2006).

Applicants submission that none of Greer, Miller, Lee or Atkinson makes up for this deficiency, is not persuasive, on the basis that as stated above, this deficiency does not exist and there is thus no need to make up for it.

Applicants further traverse the rejection on the basis that none of Greer, Atkinson, Lee or Miller in view of Liebl or Miller provides a motivation for one of ordinary skill in the art to modify a bacterial strain as claimed and the Examiner's previous assertion that such exists as taught by Greer is incorrect, as Greer is simply stating a problem and in no way proposing a solution that requires genetic engineering.

Applicant's interpreted flaws in the previously stated motivation is not found persuasive. As was previously stated, the motivation for producing a nuclease by a genetically engineered bacterial strain used in the fermentation process is to reduce the amount of nucleic acids in the medium which result in an increase in the viscosity of the medium, causing problems in the downstream processing steps, as taught by Greer et al. Greer et al. give further motivation for genetically engineering a bacterial strain to express a nuclease, because they teach that purified preparations of nucleases are expensive and

a bacterial strain that was genetically engineered to express a nuclease activity would not require an external nuclease or hydrogen peroxide to be added to the fermentation. While it is recognized that Greer et al. do not offer a solution to the problem, it is the combination of all of the references of the rejection that together offer

In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

Applicants arguments that the prior art does not lead one of ordinary skill in the art to have a reasonable success that it is possible to heterologously express a nuclease in different bacterial species, specifically *C. glutamicum* and *B. Subtilis*, wherein the nuclease gene product is secreted into the periplasmic space, which *apriori* must be a Gram positive bacteria, and released when the bacteria is lyzed by osmotic shock is also not found persuasive for the reasons previously stated and discussed above. As was stated previously, Miller et al. teach the secretion and processing of *Staphylococcal aureus* nuclease in *Bacillus subtilis*, thus the expectation of success of expressing a *Staphylococcal aureus* nuclease in Gram positive, *Bacillus subtilis* cell is high.

Applicants further argue that secondary considerations such as commercial success, long felt but unresolved needs and failure of others, etc..., support applicants position. These secondary considerations are further acknowledged but not found persuasive given the discussion previously and above.

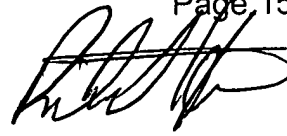
Any inquiry concerning this communication or earlier communications from the examiner should be directed to Richard G. Hutson whose telephone number is 571-272-0930. The examiner can normally be reached on M-F, 7:00-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 571-272-0928. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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A handwritten signature in black ink, appearing to read 'R. G. Hutson', with a long horizontal flourish extending to the right.

Richard G Hutson, Ph.D.
Primary Examiner
Art Unit 1652

rg
1/15/2008